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Expanding the Phenotypic Spectrum of HNRNPU-Related Disorder, Documenting the First Familial Presentation and Comprehensive Review

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ABSTRACT

HNRNPU-related neurodevelopmental disorder (*HNRNPU*-NDD) is caused by pathogenic and likely pathogenic variants in *HNRNPU*. With increasing accessibility to advanced genetic investigations, children presenting with developmental delay and intellectual disability will often undergo genomic testing; hence, the number of patients found to be affected by *HNRNPU*-NDD is increasing. We document a cohort of 17 previously unpublished patients with *HNRNPU* variants, including the first familial case, building on those previously published by our group. A comprehensive literature review was performed, identifying previously published patients and phenotypes for comparison. Eighty-four patients have been published in previous studies with pathogenic variants in *HNRNPU* with the following phenotypes: Global developmental delay, moderate to severe intellectual disability, early-onset seizures, and dysmorphic features. In addition to these phenotypes previously described, we have recognized ophthalmic abnormalities, cardiac abnormalities, and short stature in our cohort. We provide information on patients with a milder phenotype, enhancing our knowledge of phenotypic variability in *HNRNPU*-NDD.

1 | Introduction

1.1 | HNRNPU-Related Neurodevelopmental Disorder

HNRNPU-related neurodevelopmental disorder (*HNRNPU*-NDD), sometimes referred to as early infantile encephalopathy-54, was first established following the identification of a group of patients with global developmental delay (GDD) and early-onset epilepsy who all had a 1q44 microdeletion containing the *HNRNPU* gene (Caliebe et al. 2010). In addition to copy number variations, single nucleotide changes and small deletions or duplications within *HNRNPU* can lead to an overlapping phenotype (Bramswig et al. 2017; Caliebe et al. 2010; D'Cruz et al. 2013; De Kovel et al. 2016; Depienne et al. 2017; Yates et al. 2017). Since then, there have been 84 patients identified with (likely) pathogenic *HNRNPU* variants, with some recurrent variants identified, including c.1089G>A p.(Trp363*), c.706_707del p.(Glu236Thrfs*6), c.847_857del p.(Phe283Serfs*5), and c.1681dels p.(Gln561Serfs*45) (Taylor et al. 2022).

HNRNPU-NDD is caused by heterozygous variants in *HNRNPU* (MIM 602869), which encodes the heterogeneous nuclear ribonucleoprotein U (HNRNPU). The HNRNPU protein is the largest in the family of approximately 30 HNRNPs, which together comprise the most abundant proteins in the nucleus of a eukaryotic cell (Kiledjian and Dreyfuss 1992; Kletzien et al. 2023; Leduc et al. 2017). So far, proven to be expressed in the brain tissue of human fetuses and the cerebellum, liver, kidney, and heart of adult humans (Thierry et al. 2012), HNRNPU plays an important role in the development of mature neurons via alternative splicing (Fiszbein et al. 2016). This crucial role of *HNRNPU* in embryonic brain development supports the neurological and developmental abnormalities found in many patients with *HNRNPU*-NDD.

Typically, *HNRNPU* variants are de novo in etiology (Balasubramanian 2022), but we report a familial presentation for the first time here. The presentation of patients with *HNRNPU*-NDD varies; however, the common phenotypes include GDD, moderate to severe intellectual disability (ID), early-onset seizures, and craniofacial dysmorphism (Sapir et al. 2022; Schirwani et al. 2023; Song et al. 2021; Taylor et al. 2022). Common dysmorphic features identified include elongated palpebral fissures, prominent eyebrows, prominent nasal bridge, thin upper lip, and overhanging columella (Yates et al. 2017). Additionally, cardiac abnormalities have been reported in a few studies, but are considered less common with only around 30% of patients affected (Balasubramanian 2022). Of the associated cardiac issues, atrial septal defects, ventricular septal defects, and patent ductus arteriosus are seen most frequently.

With a pLI score of 1 (gnomad v4.1.0), the predominant pathogenicity mechanism associated with *HNRNPU*-NDD is haploinsufficiency (Leduc et al. 2017), supported by the majority of reports being null variants. These variants result in loss-of-function as they introduce premature stop codons resulting in mRNA products that are likely to undergo nonsense-mediated decay (Lejeune 2022). *HNRNPU* is also predicted to be intolerant to missense variants with a *Z* score of 3.5 (gnomad v4.1.0), supported by the increasing number of missense variants being reported as pathogenic. While the mechanism of loss-of-function for nonsense and frameshift variants appears straightforward, very little is known about how missense variants contribute to disease.

In this study, we provide an overview of the published literature so far including genotype–phenotype correlation for *HNRNPU*-NDD. In addition, we present data on $n = 17$ unpublished patients with *HNRNPU* variants, with particular focus on stature and cardiac abnormalities, including the first reported inherited case of *HNRNPU*-NDD.

2 | Methods

2.1 | Editorial Policies and Ethical Considerations

All participants of the study signed informed consent forms regarding the inclusion and publication of personal data and photos. The study has ethical approval with IRAS project ID:314583.

2.2 | Data Collection

This study details data from $n = 17$ probands consisting of previously unreported patients with (likely) pathogenic variants in *HNRNPU*. Data were collected using various methods and variants were classified according to the Association for Clinical Genomic Science and/or American College of Medical Genetics guidelines (Ellard et al. 2020; Firth et al. 2009; Fiszbein et al. 2016; Gillentine et al. 2021; Khadija et al. 2022; Kiledjian and Dreyfuss 1992; Kletzien et al. 2023; Leduc et al. 2017; Lee et al. 2023; Lejeune 2022; Marenda et al. 2024; Meng et al. 2023; Richards et al. 2015). All 17 patients in this cohort were collected through the *HNRNPU*-NDD natural history study either directly through the responsible clinician, HNRNP Family Foundation, or through direct contact with families. Where possible, information was primarily gathered from clinicians, but in some cases, information was obtained from parents and then supplemented from clinic letters. Two patients are reported from de-identified information from their clinicians.

3 | Results

The phenotype details of our cohort are summarized in the following 17 probands. Further details regarding pregnancy, developmental milestones, medical history, and dysmorphism can be found in Table 1 and Figure 1A–C. The phenotype findings of the 12 previous papers detailing patients with *HNRNPU* variants found in a review of the literature are displayed in Table 2. Table 1 depicts the genotype–phenotype correlations of our 17 patients.

3.1 | Proband 1

Patient 1 was a female aged 22.84 years who was in the < 0.4th percentile for height and the 75th to 91st percentile for weight.

TABLE 1 | Genotype–phenotype data from our cohort—Patients 1–17.

Proband number	Variants	Neonatal feeding difficulties	Intellectual disability	Autistic features	Hypotonia	Seizures	Brain scan abnormalities	Cardiac abnormalities	Short stature
1	c.1137dup	−	−	−	−	+	−	−	+
2	c.2304_2305del	−	+	−	−	+	−	−	−
3	c.481C>T	−	+	−	−	+	+	+	+
4	c.2217_2218del	+	+	−	+	+	−	−	−
5	c.669_691dup	−	−	−	−	+	−	−	−
6	c.520C>T	−	+	−	−	+	−	+	−
7	c.565G>T	−	+	−	−	−	−	−	−
8	c.803+2T>C	+	+	−	−	+	+	+	+
9	c.804-9_804-6del	+	+	+	+	+	−	−	−
10	c.311_312insA	+	+	+	+	+	+	−	+
11	c.1352A>C	+	−	+	+	+	+	+	+
12	c.1961A>G	+	+	−	+	+	−	−	−
13	c.1765del	−	+	+	+	+	+	−	−
14	c.2425-3C>G	+	+	+	+	+	+	−	−
15	c.1230+4A>G	+	+	−	+	+	+	−	+
16	c.2270_2271del	−	+	−	−	+	−	−	−
17	c.804-9_804-6del	+	+	+	+	+	+	−	+

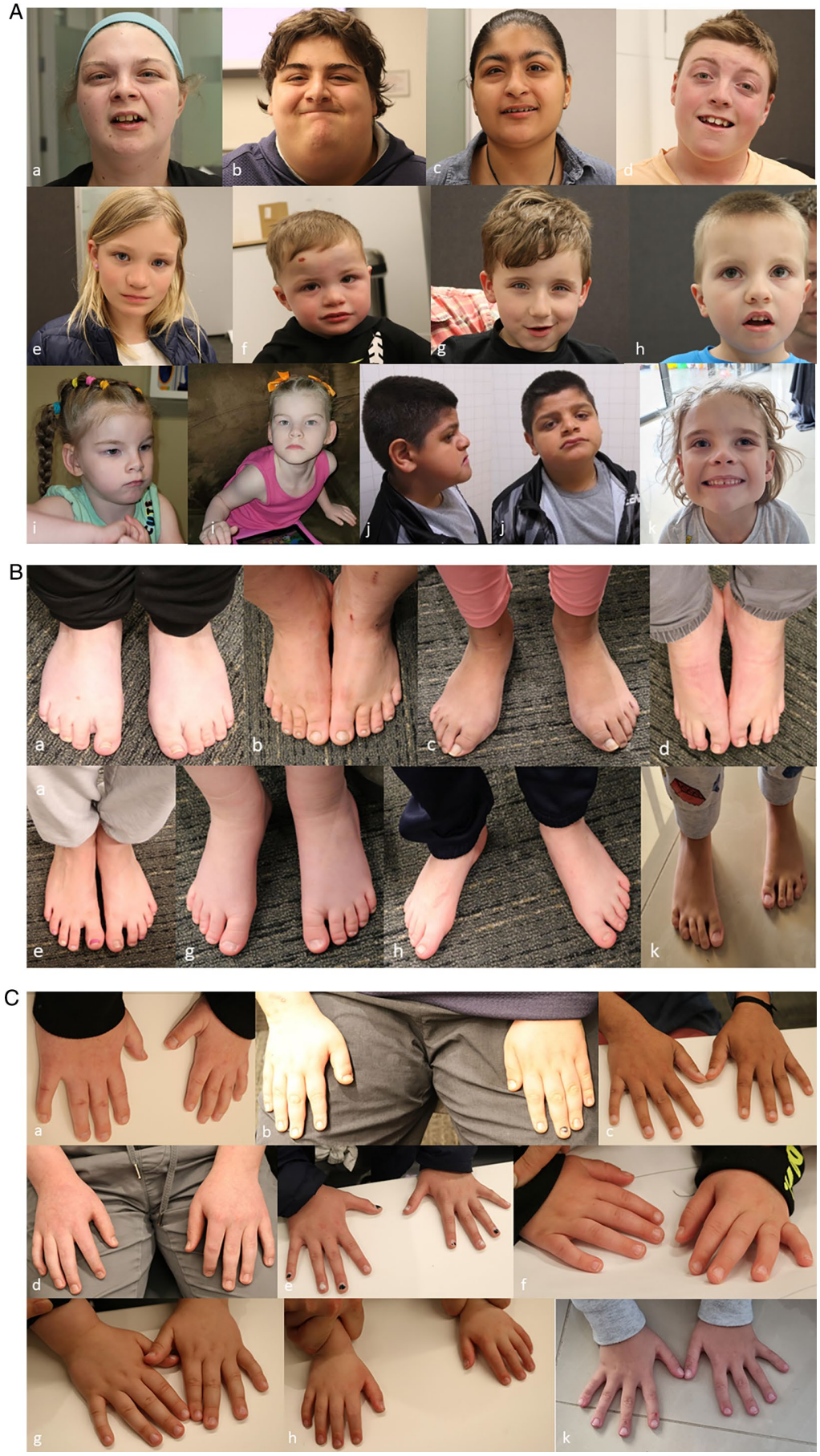

FIGURE 1 | Legend on next page.

Developmental delay was noted at 6–9 months of age. She sat unsupported at 12 months, took her first steps at 24 months, and said her first words at the age of 3 years. She is conversant and engaging. At 16 months of life, tonic seizures and febrile seizures began, which were well controlled with medication. However, she also experienced stress-induced cluster myoclonic seizures. No cardiac abnormalities had been detected. She had limitations to supination of forearms and small hands and feet. A pathogenic *HNRNPU* frameshift variant (NM_031844.3:c.1137dup, p.(Gly380Trpfs*13)) was identified, with presumed de novo inheritance, although parental testing was not performed.

3.2 | Proband 2

Patient 2 was a male aged 15.02 years who was in the 9th to 25th percentile for height and the >99.6th percentile for weight. A developmental delay and ID were noted: sitting at 6–9 months, crawling at 12 months, walking at 18 months, and saying his first words at 1 year. He was conversant to an extent, and perseverative. Complex febrile seizures occurred from 3 years until 7 years of age. There was no history of cardiac abnormalities. He was born with unilateral cryptorchidism and micropenis. He also had limitations to supination of forearms. A de novo frameshift *HNRNPU* variant (NM_031844.3:c.2304_2305del, p.(Gly769Glufs*83)) was identified.

3.3 | Proband 3

Patient 3 was a female aged 15.33 years who was in the second percentile for height and the <0.4th percentile for weight. GDD and ID were noted. She walked at 22 months and said her first words at 2 years. Absence seizures began in her second year of life, followed by febrile seizures. At 16–17 months, generalized tonic–clonic seizures began and status epilepticus occurred. An electroencephalogram (EEG) showed seizure activity, and a brain MRI showed syringomyelia. The cardiac assessment discovered a ventricular septal defect. A nonsense *HNRNPU* variant (NM_031844.3:c.481C>T p.(Gln161*)) was identified with presumed de novo inheritance, although parental testing was not performed.

3.4 | Proband 4

Patient 4 was a male aged 14.99 years who was in the 9th to 25th percentile for height and the 75th to 91st percentile for weight. He had an ID and was 12 months delayed developmentally, with delayed speech and not walking until 23 months of age. He was conversant and engaging. Febrile seizures started at 20 months of age. The EEG showed a spike wave pattern, and the brain MRI was reportedly normal. No cardiac issues had been detected. A pathogenic frameshift *HNRNPU* variant was identified (NM_031844.3:c.2217_2218del, p.(Gly740Trpfs*24)), with presumed de novo inheritance, although parental testing was not performed.

3.5 | Proband 5

Patient 5 was a female aged 9.23 years who was in the 9th to 25th percentile for height and the 91st percentile for weight. No developmental delay or ID was noted; she had no language issues and took her first steps at 11 months old. Absence seizures started at 5 years of age, with one generalized tonic–clonic seizure at 7 years and a queried febrile seizure. Her EEG was reported abnormal, consistent with the absence of seizures. Brain MRI was normal. No cardiac abnormalities had been detected. The paternally inherited missense variant in *HNRNPU* was NM_031844.3: c.669_691dup p.(Gly231Valfs*116). No clinical abnormalities were reported in the father.

3.6 | Proband 6

Patient 6 was a male aged 3.82 years who was in the 2nd to 9th percentile for height and the 50th percentile for weight. He had a GDD, was able to say two words joined together, and took his first steps at 20 months. He had a diagnosis of Doose syndrome (myotonic–atonic seizures), with previous febrile seizures. Both EEG and brain MRI were normal. A ventricular septal defect was detected and repaired at 5 months of age. A de novo nonsense variant in *HNRNPU* was identified (NM_031844.3:c.520C>T p.(Gln174*)).

3.7 | Proband 7

Patient 7 was a male aged 5.40 years who was in the 91st percentile for height and the 98th to 99.6th percentile for weight. Developmental delay was noticed, specifically regarding vision and language, and he had ID. He was nonverbal and took his first steps at 18 months. The cardiac assessment detected no abnormalities, and he had never experienced any seizures. His brain MRI was unremarkable. A de novo *HNRNPU* missense variant (NM_031844.3:c.565G>T, p.(Gly189Cys)) was identified.

3.8 | Proband 8

Patient 8 was a male aged 5.51 years who was in the 0.4th to 2nd percentile for height and the 9th to 25th percentile for weight.

FIGURE 1 | (A) Dysmorphic features of the face. Patients in presented cohort: (a) Patient 1 (22 years), (b) Patient 2 (15 years), (c) Patient 3 (15 years), (d) Patient 4 (14 years), (e) Patient 5 (9 years), (f) Patient 6 (3 years), (g) Patient 7 (5 years), (h) Patient 8 (5 years), (i) Patient 9 (5 years), (j) Patient 10 (13 years), and (k) Patient 11 (8 years). Dysmorphic features including varying palpebral fissure abnormalities, strabismus, prominent nasal bridge, thin upper lip, frontal bossing, and low set ears. (B) Dysmorphic features of the feet. Patients in presented cohort: (a) Patient 1, (b) Patient 2, (c) Patient 3, (d) Patient 4, (e) Patient 5, (g) Patient 7, (h) Patient 8, and (k) Patient 11. Varying foot abnormalities including clinodactyly, short toes, long hallux, short feet, and narrow feet. (C) Dysmorphic features of the hands. Patients in presented cohort: (a) Patient 1, (b) Patient 2, (c) Patient 3, (d) Patient 4, (e) Patient 5, (f) Patient 6, (g) Patient 7, (h) Patient 8, (i) Patient 9, and (k) Patient 11. Hand abnormalities including short hands, clinodactyly, and tapered digits.

TABLE 2 | Phenotype data from literature review.

	Our study	Caliebe et al. (2010)	Thierry et al. (2012)	Allen et al. (2013)	De Kovel et al. (2016)	Bramswig et al. (2017)	Depienne et al. (2017)	Leduc et al. (2017)	Yates et al. (2017)	Durkin et al. (2020)	Gillentine et al. (2021)	Song et al. (2021)	Taylor et al. (2022)	Khadija et al. (2022)	Overall in current literature
Neonatal hypotonia	9/15	n/r	n/r	n/r	n/r	2/7	n/r	1/4	2/7	1/21	n/r	n/r	11/17	n/r	17/56 (30.36%)
Neonatal feeding difficulties	9/15	n/r	n/r	n/r	n/r	3/7	n/r	n/r	1/7	8/21	n/r	n/r	10/17	n/r	22/52 (42.31%)
Intellectual disability	14/17	4/4	11/11	1/1	n/r	6/6	7/7	4/4	7/7	11/21	40/45	1/2	16/17	1/2	109/127 (85.83%)
Global developmental delay	15/17	4/4	11/11	1/1	1/1	4/6	7/7	4/4	7/7	19/19	4/32	1/2	17/17	1/2	81/113 (71.68%)
Autistic features	6/17	n/r	4/9	1/1	n/r	3/7	1/7	1/4	1/5	3/21	19/36	1/2	8/16	n/r	42/108 (38.88%)
Hypotonia	9/15	4/4	4/9	n/r	1/1	6/7	4/5	3/4	0/7	6/21	8/30	n/r	15/17	1/2	52/107 (48.60%)
Seizures	16/17	4/4	11/11	1/1	1/1	6/7	6/7	4/4	6/7	20/21	35/46	2/2	17/17	1/2	114/130 (87.69%)
Seizures before 2 years	8/12	2/3	n/r	1/1	1/1	3/6	4/6	2/4	2/6	3/21	n/r	0/2	6/14	n/r	24/64 (37.50%)
Brain MRI/CT abnormality	8/14	4/4	8/11	1/1	1/1	5/6	4/6	3/4	2/7	6/21	5/30	0/2	6/12	2/2	47/107 (43.93%)
Ophthalmic abnormalities	12/17	2/4	4/8	n/r	n/r	4/7	n/r	2/4	1/7	5/20	7/32	n/r	5/15	n/r	30/97 (30.93%)
Dysmorphic features	17/17	4/4	11/11	0/1	1/1	7/7	n/r	4/4	6/7	20/21	7/32	2/2	16/17	2/2	80/109 (73.39%)
Cardiac abnormality	4/13	2/4	1/11	n/r	n/r	4/6	n/r	0/4	1/7	3/21	3/33	2/2	7/16	1/2	24/106 (22.64%)
Short stature	7/16	2/4	n/r	n/r	0/1	4/7	3/6	1/4	4/7	5/21	4/31	0/1	1/1	n/r	24/83 (28.92%)

Developmental delay and ID were noted; he said his first words at the age of 3.5 years but had poor communication, sat unsupported at 1 year, and walked at 2.5 years. Between 4 and 12 months of age, apneic seizures and febrile seizures occurred. His EEG was normal, with no seizures occurring during the investigation. Brain MRI found mild delayed myelination. A cardiac assessment identified an atrial septal defect. A splice-disrupting variant in *HNRNPU* (NM_031844.3: c.803+2T>C) was identified with presumed de novo inheritance, although parental testing was not performed.

3.9 | Proband 9

Patient 9 was a female aged 5.88 years who was in the 2nd to 9th percentile for height and the 9th to 25th percentile for weight. She had GDD and severe ID. She was mainly nonverbal and had a receptive expressive language disorder, and she took her first steps at 2 years. There were suspected absence seizures. The 1-h EEG showed multifocal epileptiform discharges which often appeared diffuse, superimposed on a mildly slow background. Brain MRI had no abnormal findings. There was no evidence of any cardiac abnormalities. A variant predicted to affect mRNA splicing in *HNRNPU* (NM_031844.3:c.804-9_804-6del) was identified, with presumed de novo inheritance.

3.10 | Proband 10

Patient 10 was a male aged 13 years who was in the < 0.4th percentile for height and the 9th to 25th percentile for weight. He had GDD, severe ID, and autism spectrum disorder (ASD). He is essentially nonverbal with only three words at present, and took his first steps at 2 years. At 8 months, seizures began, at first in the context of a fever and then spontaneously. Seizures ceased at age 7. The EEG reported generalized intermittent epileptiform activity, focal epileptiform activity in the left temporal and frontal regions, and background slowing. A brain MRI revealed a middle cranial fossa subarachnoid cyst. He had no history of cardiac abnormalities. A frameshift variant in *HNRNPU* (NM_031844.3:c.311_312insA, p.(Met104Ilefs*41)) was identified, with presumed de novo inheritance.

3.11 | Proband 11

Patient 11 was a male aged 8.15 years who was in the 0.4th to 2nd percentile for height and the 9th to 25th percentile for weight. He had mild developmental delay. There was a language delay, however his speech was age appropriate. He sat unsupported at 1 year, and took his first steps at 2 years of age. At 3 years of age, a singular febrile seizure occurred, resolving within a short period of time. A brain MRI detected mild symmetrical thinning of the corpus callosum with no focal lesion, and nonspecific mild symmetric ventriculomegaly of bilateral lateral, and third ventricles (likely consistent with mild cerebral atrophy). Cardiac abnormalities included a small patent ductus arteriosus and patent foramen ovale at birth, both of which closed spontaneously. A de novo *HNRNPU* missense variant (NM_031844.3:c.1352A>C, p.(His451Pro)) was identified.

3.12 | Proband 12

Patient 12 was a male aged 7.08 years who was in the 9th to 25th percentile for height and on the 91st percentile for weight. He had GDD and severe ID. He walked unaided at age 28 months and experienced language regression, now being nonverbal. At 8 months of life, seizures began, first in the context of fever and then spontaneously. An interictal EEG consisted of slow background activity and very frequent spike and wave discharges over the frontal regions bilaterally. The brain MRI was unremarkable. He had no history of cardiac abnormalities. A de novo missense variant in HNRNPU (NM_031844.3:c.1961A>G p.(Tyr654Cys)) was identified.

3.13 | Proband 13

Patient 13 was a female aged 15.38 years. She had GDD, ID, and ASD. Her past medical history included refractory epilepsy. The brain MRI showed possible cortical dysplasia. No cardiac abnormalities had been detected. A de novo frameshift variant in *HNRNPU* (NM_031844.3:c.1765del, p.(Gln589Argfs*17)) was identified.

3.14 | Proband 14

Patient 14 was a male aged 9.48 years who was at the 15th percentile for height. He had GDD, ID, and ASD. He had a marked delay in expressive speech with no meaningful words and took his first steps at 20 months. He first experienced seizures at 2 years of age, initially associated with febrile illnesses. He had epilepsy with a Lennox–Gastaut phenotype- experiencing intermittent myoclonic drops. These events were thought to be atypical absences, of which he experienced up to 20 a day. His EEG was reportedly normal. A brain and spinal MRI showed symmetrical T2 hyperintensities in the subcortical white matter of the basal frontal lobes bilaterally on the coronal T2 space imaging. When compared to the previous MRI, there was slightly increased prominence of the cerebral sulci and cerebellar folia raising the possibility of mild volume loss. There was no history of cardiac abnormalities. A de novo variant in *HNRNPU* (NM_031844.3:c.2425-3C>G) was identified, which is predicted to affect mRNA splicing.

3.15 | Proband 15

Patient 15 was a male aged 3 years who was in the 0.4th to 2nd percentile for height and on the 25th percentile for weight. He had GDD and moderate ID. He sat unsupported at 14 months and said his first words at 7 months, but then became nonverbal. He had focal seizures with secondary generalization. An EEG showed marked excess of high amplitude rhythmic delta slowing, some discrete generalized spike–wave discharges, and some discrete sharp-slow complexes. The brain MRI reported delayed

myelination. There was no evidence of cardiac abnormalities. A de novo variant in *HNRNPU* (NM_031844.3:c.1230+4A>G) was identified and is predicted to affect mRNA splicing.

3.16 | Proband 16

Patient 16 was a female aged 6.17 years. Her measurements were last reported at 3.5 years, when she was in the 25th to 50th percentile for height and the 25th percentile for weight. She had GDD and ID. Seizures (myoclonic epilepsy and generalized tonic–clonic seizures) began at 25 months. There was no evidence of cardiac abnormalities. A frameshift variant in *HNRNPU* (NM_031844.3:c.2270_2771del, p.(Pro757Argfs*7)) was identified, with presumed de novo inheritance, although parental testing was not performed.

3.17 | Proband 17

Patient 17 was a male aged 9.17 years who was in the 0.4th percentile for height and the 25th percentile for weight. He had GDD, ID, and ASD. He babbled between 4 and 9 months of age; however, this stopped and he became nonverbal. Generalized tonic–clonic seizures and myoclonic jerks began at 2 years of age, with an EEG showing background slowing. Brain MRI reported bilateral ventriculomegaly. There was no history of cardiac abnormalities. A variant predicted to affect mRNA splicing in *HNRNPU* (NM_031844.3:c.804-9_804-6del) was identified, with presumed de novo inheritance.

4 | Discussion

4.1 | Protein Structure and Function

HNRNPU regulates gene expression in various ways, it is crucial in chromatin remodeling and plays a large role in RNA processing of many genes, including some transcription factors (Balasubramanian 2022; Kiledjian and Dreyfuss 1992; Sapir et al. 2022). In fact, the role of HNRNPU in gene expression is so crucial that our team and others were recently able to show that *HNRNPU* variants have a unique methylome (Lee et al. 2023). HNRNPU is composed of 825 amino acids with four main domains, each critical for the protein function (Figure 2A) (Firth et al. 2009). The SAP domain allows HNRNPU to bind DNA, which is crucial for its role in maintaining chromatin organization (Aravind and Koonin 2000). The SPRY domain is important for protein–protein interactions; it is involved in various signaling pathways that are important for chromatin regulation (D'Cruz et al. 2013). The PNK domain of HNRNPU is responsible for ATP binding required for self-assembly (Kletzien et al. 2023; Marenda et al. 2024). Finally, the unstructured C-terminal domain (CTD) contains multiple RGG boxes and is involved in RNA binding, which is necessary for HNRNPU's role in RNA processing (Kletzien et al. 2023). An AlphaFold3 prediction of HNRNPU structure (Figure 2A) shows the SPRY and PNK regions in close contact, forming one globular fold (Abramson et al. 2024). The inclusion of ATP and Mg^{2+} in the prediction highlights the importance of the PNK region in binding these molecules.

4.2 | Genotype

Upon analysis of the included variants and comparison with those previously published, we identified a genotype recurrence in two of our patients, Probands 9 and 17, with the heterozygous cDNA change at c.804-9_804-6del. This was the same variant found in a patient by Taylor et al. (2022) and is present in ClinVar (Variation ID: 1685882). Phenotypic similarities between these two patients included neonatal feeding difficulties (swallowing problems), ID, GDD, language delay, motor delay, autistic features, and dysmorphic features.

A spectrum of variants in *HNRNPU* results in pathogenicity (Figure 2B). The most common being frameshift variants, which occurred in 51.52% of patients with *HNRNPU*-NDD. In descending order of incidence, other variants were seen as nonsense, missense, splice, and finally, in-frame deletions in only 2 patients. The position of the variants occurred among most exons in *HNRNPU*, except Exons 8 and 13, with the most common sites being Exons 1, 9, and 10.

4.3 | Discussion of Phenotypes

Reviewing the literature, 84 patients with *HNRNPU* variants were identified (Allen et al. 2013; Bramswig et al. 2017; Caliebe et al. 2010; De Kovel et al. 2016; Depienne et al. 2017; Durkin et al. 2020; Gillentine et al. 2021; Khadija et al. 2022; Leduc et al. 2017; Song et al. 2021; Taylor et al. 2022; Thierry et al. 2012; Yates et al. 2017). Recurring clinical features of these patients were GDD, ID, seizures, and craniofacial dysmorphism. Among the new data presented here and the other patient data collected, which includes earlier work by our group, we are able to further our understanding of *HNRNPU* variants and view a milder phenotype emerging (Durkin et al. 2020; Taylor et al. 2022; Yates et al. 2017).

4.4 | Familial Inheritance

All previous data of *HNRNPU*-NDD have described de novo mutations as the pattern of inheritance. One of our patients was found to have the same variant, c.669_691dup p.(Gly231Valfs*116), as her father. This is the first reported case of germline inheritance in the literature, although one variant has been reported as paternally mosaic patient H1, NM_031844.3:c.16delinsATT, p.(Val6Ilesfs*4) (Depienne et al. 2017). The patient, Proband 5, was the only case in the cohort who was neither developmentally delayed nor had ID. Other distinctions between her and many with *HNRNPU*-NDD, were her stature being in the normal range, having no cardiac abnormalities, and a normal brain MRI. However, similar to a large proportion of patients, she experienced absence and generalized tonic–clonic seizures- with an abnormal EEG. Her father had no reported clinical features, suggesting there may be incomplete penetrance. The diagnoses were made using a targeted epilepsy panel containing 308 genes. While this panel is comprehensive, it is possible that other genetic factors may be responsible for the severity in the child. However, it is now well-recognized that neurodevelopmental disorders may present in the context of an inherited variant with variable phenotypes. Similarly, other rare genetic disorders have

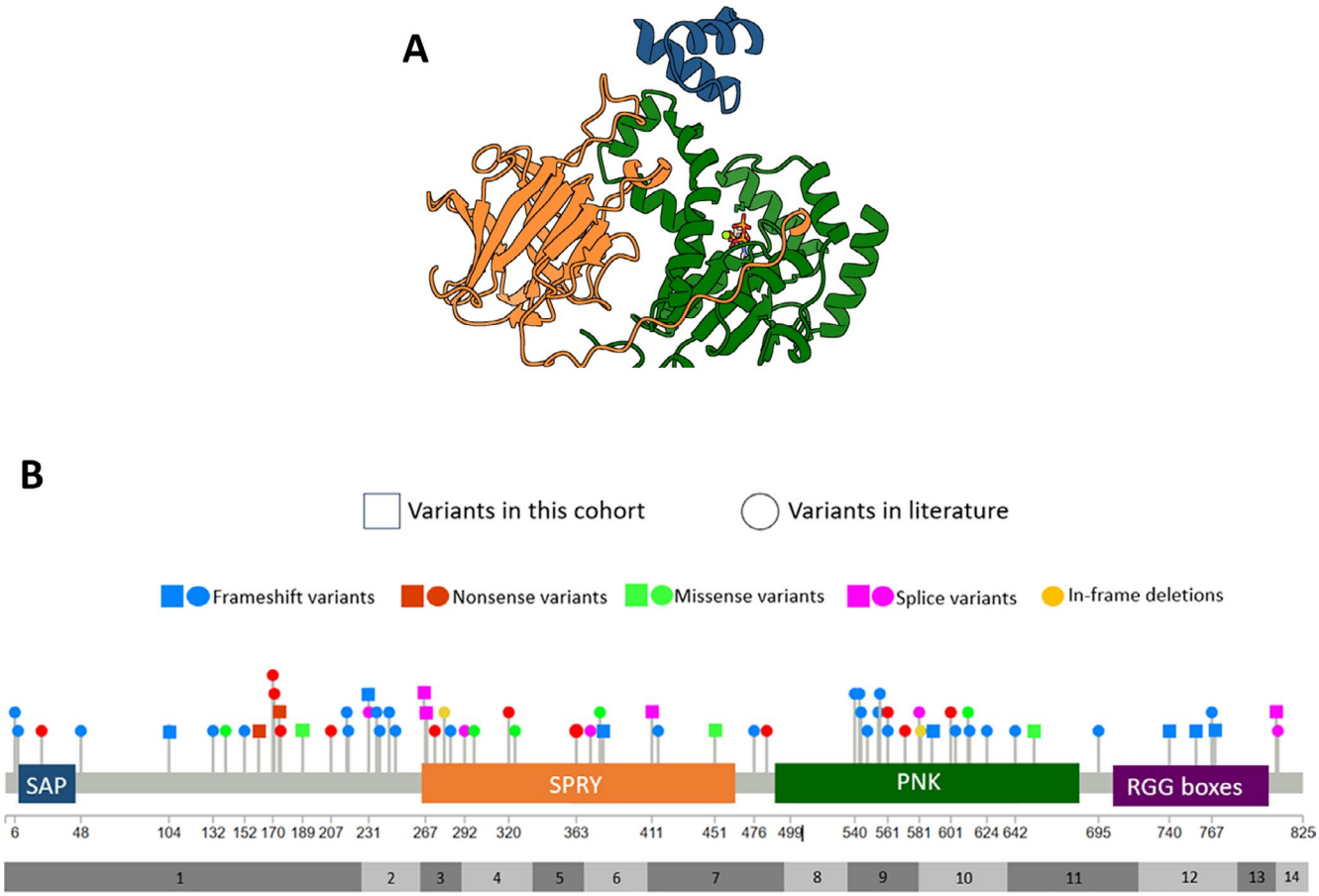

FIGURE 2 | (A) 3D prediction of human HNRNPU protein structured domains bound to ATP and Mg^{2+}. Predicted structure of HNRNPU created with chimeraX (Abramson et al. 2024). Based on AlphaFold 3 prediction from *HNRNPU* sequence (Allen et al. 2013). The domains of HNRNPU are highlighted based on positional information cited by Kletzien et al. (2023). SAP (green), SPRY (red), and PNK (blue). Mg^{2+} is depicted by the green ball and ATP is shown adjacent, bound to PNK. Unstructured regions outside of functional domains are not shown as pIDDT confidence levels for these regions are very low. (B) 2D schematic of HNRNPU protein domains with variant interpretation plot of all known pathogenic *HNRNPU* variants. Schematic of unstructured HNRNPU showing all the domains of the protein including the unstructured C-terminal domain containing the RGG boxes. This representation has been used to create a lollipop diagram of all *HNRNPU* variants known to result in disease with those in literature represented as a circle, and those reported in this review represented as a square.

been identified to exhibit a wide range of clinical presentations, even within family members sharing the same genetic variation (Schirwani et al. 2023). Theorizing an asymptomatic or extremely mild phenotype, our group has previously shown in another rare NDD, ASXL3-related disorder, pathogenic variants can present with a severe phenotype in the child, and a seemingly unaffected parent (Schirwani et al. 2023), mirroring the variability observed in Proband 5 of the current study. In the future, as more patients with familial inherited *HNRNPU*-NDD are reported, we will be better able to decipher any patterns of differences between variants with differing inheritance.

4.5 | Craniofacial Abnormalities

Our research identified dysmorphic features in 100% (17 out of 17), in accordance with the 94.81% formerly reported. Across all studies, the incidence of nondysmorphism has been a maximum of one patient per study. A considerably broad range of features has been recorded, summarizing that no characteristic appearance has yet been achieved. Our data did, however, show repetition of the following features: abnormal palpebral fissures in 52.94% (9 out of 17) and a thin upper lip in 64.71% (11 out of 17). As previously experienced, the clinical information of five of our patients was collected from the parents—mainly from description (Taylor et al. 2022) suggesting some limitation to the analyses.

Other clinical features identified in the literature were ophthalmic abnormalities, previously reported in about 30% of patients (Gillentine et al. 2021). Again, we viewed a higher proportion with 70.59% of patients (12 out of 17). The most frequent abnormality is strabismus, experienced by six patients in our cohort, and reported collectively nine times in previous studies (Durkin et al. 2020; Taylor et al. 2022; Yates et al. 2017).

4.6 | Seizures and Brain Imaging

A very widely reported phenotype of patients with *HNRNPU* variants is seizures. All previous studies described this, with a significant incidence of 94.05%. Our data further accentuates

this with 94.12% (16 out of 17) having experienced seizures, with 8 experiencing febrile seizures. Early-onset seizures were common in our cohort, with 66.67% (8 out of 12) having seizures before 24 months of age. This was substantially higher than the 37.5% reported in the literature. Similar to previous studies, we had a minimal number of EEG reports, despite a large proportion of patients experiencing seizures. From 11 EEGs reported, we noted a wide-ranging seizure phenotype. The most common seizure type experienced was generalized tonic–clonic seizures, followed closely by absence seizures, identical to the results previously described (Taylor et al. 2022).

Brain MRI abnormalities were found in just over half of our patients (57.14%, 8 out of 14), noting that three had not undergone an MRI, so they cannot be ruled as normal. Likewise, 54.55% of previously published patients had detectable abnormalities. The irregularities were all distinct, with no repetitions in our data.

4.7 | Intellectual and Developmental Delay

Of the characteristic clinical features described by Balasubramanian (2022), ID and developmental delay were present in the majority of patients. Our data showed 82.35% of patients (14 out of 17) had ID and 88.24% (15 out of 17) had GDD. This was in keeping with previous literature, which identified 84.15% with ID and 95.06% with GDD. As detailed in the literature, patients with *HNRNPU* variants have varying severities of language delay. Our cohort ranged from no language delay, to being able to say a couple of words, to six of our patients being essentially nonverbal—thus at the more severe end of the scale. There was only a single patient we found to not have an ID, motor delay, or language delay.

A diagnosis of ASD was present in four of our patients, with autistic features being recognized in 35.29% (6 out of 17)—the final two patients without a formal ASD diagnosis are currently under investigation. This was consistent with the rate in the literature of 31.95%. Less common, but still prevalent, was a diagnosis of ADHD (17.65%, 3 out of 17).

4.8 | Neonatal Difficulties

Upon reviewing the literature, a difference between the rate of observations of hypotonia during the neonatal period was identified. We viewed 60% of patients (9 out of 15) having experienced hypotonia, in comparison to 30.36% in the literature. More comparable statistics were however found for neonatal feeding difficulties: 60% of our patients (9/15), vs. 42.31% in previous studies.

4.9 | Growth

Our previous work suggested that short stature may be prevalent in patients with *HNRNPU*; hence, we looked at growth parameters specifically in this cohort. Our data showed that 43.75% (7 out of 16) fit the criteria and thus were classed as having short stature. A similar statistic of 38.46% was concluded by calculation of the literature statistics. This rate may be due to the high incidence of feeding difficulties or the developmental delay seen in *HNRNPU*-NDD. No studies have investigated causative factors; thus, the root of the short stature is a potential area of future research, with the aim of better management from an earlier age. One patient had obesity, but the rest had an average weight. All head circumferences were in the normal average range.

4.10 | Cardiac Defects

We focused on exploring whether cardiac abnormalities are related to the *HNRNPU* variants, finding 30.77% of patients (4 out of 13) affected. Most abnormalities were septal defects, with two ventricular septal defects, one atrial septal defect, and one patent foramen ovale; there was one patent ductus arteriosus. Two of the defects closed spontaneously, while one ventricular septal defect required surgical intervention. Previously, 28.77% of patients identified with a variant in *HNRNPU* were found to have cardiac abnormalities. However, three of the previous studies did not comment on this clinical feature. Taylor et al. (2022) also reported septal defects in their cohort. The proportion of patients experiencing cardiac abnormalities was lower than expected; however, there is scope for further understanding of a causal link between variants in *HNRNPU* and congenital heart defects, as mouse models have shown HNRNPU is important in cardiac development (Ye et al. 2015).

4.11 | Conclusion

In this study, we further the knowledge of the phenotype of *HNRNPU*-NDD. Building upon the 84 patients published to date, we added 17 new patients. Documenting the first ever reported inherited case, we create the beginning of a base of knowledge to allow comparison of de novo and familial inherited *HNRNPU* phenotype and genotype data. To further the understanding of the progression and natural history of *HNRNPU*-NDD, future patients diagnosed at a later age need to be considered.

Through this study, we have expanded the phenotype of *HNRNPU*-NDD, which is crucial in aiding the accurate diagnoses of future patients, especially as a milder phenotype is becoming apparent. Through focusing on cardiac abnormalities, we have seen a markedly higher proportion of patients affected than in the literature. The lower occurrences previously seen may be attributed to a lack of investigation and reporting in many of the patients that were previously published. It would be useful to compare additional echocardiogram data in further patients with and without detected cardiac abnormalities, to confirm if the pattern of septal defects we have observed is a true finding. Hence, adding further evidence to the idea proposed by our group, that patients with *HNRNPU* variants should be offered echocardiograms as a form of cardiac monitoring to aid in patient management and diagnosis (Taylor et al. 2022). The observation of cardiac abnormalities is consistent with data from a mouse conditional *HNRNPU* knockout in the heart which showed that HNRNPU is required for normal postnatal heart development (Ye et al. 2015). Further, we have highlighted other areas for potential research through focus on rarer reported phenotypes such as short stature.

By broadening the genotype–phenotype correlation of *HNRNPU* and the location of variants being mainly in Exons 1, 9, and 10, we strengthen the evidence highlighting the importance of looking into alternative *HNRNPU*-NDD treatments, such as targeted gene therapy which has the potential to restore the nonfunctional HNRNPU protein.

Author Contributions

M. Balasubramanian conceived the study, collated patient samples, collected clinical data, and supervised the project. A. K. O. Hodgson collected clinical data and wrote the manuscript. L. Baxandall wrote the manuscript and made Figure 2A,B. R. Sánchez-Carpintero collected clinical data. R. Sidlow collected clinical data. H. Goel collected clinical data. All authors critically reviewed and accepted the final manuscript.

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Ethics Statement

HNRNPU-related neurodevelopmental disorder: Creating an international registry and natural history study. REC reference: 22/NE/0125 IRAS project ID: 314583 (North East–Newcastle & North Tyneside 2 Research Ethics Committee).

Consent

All participants of the study signed informed consent forms regarding the inclusion and publication of personal data and photos.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available on request from the authors (subject to patient consent).

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